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Sustainability Spotlight:

This study advances sustainable packaging by creating biodegradable starch/PVA–PEG copolymer films infused with green tea extract (TE). By replacing petroleum-based plastics with renewable, compostable materials, the films reduce plastic pollution and dependence on fossil resources. Their natural antioxidant and antibacterial properties extend food shelf life, decreasing food waste. This innovation directly supports UN Sustainable Development Goals 12 (Responsible Consumption and Production), 13 (Climate Action), and 14 (Life Below Water) by promoting circular material use, mitigating environmental impact, and protecting ecosystems from plastic contamination. Overall, the work exemplifies a holistic approach to developing eco-friendly packaging solutions for a more sustainable future.

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Development of Multifunctional and Sustainable Starch/Polyvinyl Alcohol–Polyethylene Glycol Copolymer Films Reinforced with Green Tea Extract for Food Packaging

Neha Rana[a,b], Ruchika[a,b], Ankit Saneja[a,b*]

[a]Formulation Laboratory, Dietetics and Nutrition Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur-176061, Himachal Pradesh, India

[b]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad – 201002, India

***Corresponding Author**
Dr. Ankit Saneja
Senior Scientist
Dietetics and Nutrition Technology Division,
CSIR – Institute of Himalayan Bioresource Technology,
Palampur, 176061
Himachal Pradesh, India
E-mail: ankit.saneja@csir.res.in; ankitsaneja.ihbt@gmail.com
Tel: 91-1894-233339, Ext: 485

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Abstract

This study explores a sustainable alternative to conventional plastic packaging by developing biodegradable films from starch, polyvinyl alcohol–polyethylene glycol (PVA-PEG) copolymer and green tea extract (TE) as functional additive. The incorporation of TE at 0.5 % w/v demonstrated to significantly enhance tensile strength (4.7 ± 0.3 MPa) and water contact angle (70.7 ± 0.3°) in comparison to blank STKB film. The developed films also demonstrated significant improvement in barrier attributes of the film including, UV-shielding (100%), water vapour and oxygen transmission. Further, the films were analysed *via* several techniques, including Scanning Electron Microscopy (SEM), 3D optical profilometry, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD) and Thermogravimetric Analysis (TGA). The results demonstrated that incorporating TE improved the structure, intermolecular interactions and thermal stability of the film. The DPPH assay and cytocompatibility (95 %) in L929 fibroblast confirmed the strong antioxidant and biocompatibility of the developed film. The incorporation of TE enhanced the antibacterial potential of the films, with significant inhibition of *Escherichia coli* and *Staphylococcus aureus*. The preservation application of developed films on fresh cut apple cubes demonstrated reduced browning index, weight loss and pH indicated better preservation compared to the blank film. Finally, the biodegradability of the film was assessed by soil burial tests demonstrated residual area (99.35 ± 0.64) within 10 days. These results highlight the potential of ST/PVA-PEG/TE films as eco-friendly, functional packaging materials to improve food shelf life while ensuring safety and sustainability.

Keywords: Biodegradable films, green tea extract, Starch/PVA-PEG, Antioxidant properties, Antibacterial activity, Fresh-cut apple preservation

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1. Introduction

Increasing concerns about plastic pollution have intensified the quest for sustainable packaging solutions for food preservation while being environmentally friendly[1, 2]. Traditional petroleum-based plastics remain in the environment for decades, collecting in landfills and waterways, which has sparked interest in renewable and biodegradable options that align with circular-economy principles. Biopolymer films are particularly noteworthy as they can provide food protection while minimizing long-term environmental impact when properly disposed off [3, 4]. Starch, an abundant and cost-effective polysaccharide, is a primary focus for biodegradable food packaging due to its effective film-forming capacity and natural composability. Starch-based films are widely used in different food applications due to their desirable features, such as high transparency, good sensory qualities, and excellent gas barrier properties [5, 6]. However, their broader application in food packaging is hindered by drawbacks like low water resistance and weak mechanical strength. A promising approach to enhance their performance is to develop blend films by incorporating starch polymers with other compatible polymers [7-9].

By blending starch with other polymers, the films may gain enhanced strength and integrity, while the addition of polyethylene glycol (PEG) serves as a plasticizer, improving flexibility and allowing for better control over water interactions [7, 10, 11]. To further enhance functionality beyond simple physical protection, researchers are increasingly integrating natural bioactive into biopolymer matrices. Green tea extract (TE), rich in catechin polyphenols, is recognized for its antioxidant and antimicrobial properties, making it effective in combating oxidation and inhibiting foodborne pathogens[12, 13]. The incorporation of TE into biodegradable films has shown to boost radical-scavenging activity and provide antibacterial benefits essential for extending shelf life and ensuring food safety [13-16].

Therefore, this study focuses on developing a starch/PVA-PEG copolymer film that incorporates TE, aiming to combine biodegradability with inherent antioxidant and antibacterial functions. In this study, we have blended PVA-PEG copolymer with starch and TE. The main objective was to present a sustainable alternative to conventional plastics that also actively contributes to maintaining food quality. Further, instead of utilizing an external plasticizer, PVA-PEG copolymer was utilized, which has intrinsic

plasticizing properties. This approach provides the advantage of eliminating extra optimization step needed for external plasticizer to avoid leaching phenomenon in the films. Further, PVA-PEG copolymer is well known for its excellent film forming ability and commonly utilized in pharmaceutical industries [17]. Beyond functional improvements, the selection of bioactive ingredients and polymer blends considered biocompatibility and safety for food contact, which is crucial for packaging. A thorough characterization investigated based on mechanical and surface properties was carried to analyse the influences of TE on the structure and properties of the films. The morphology and surface topography was analysed using scanning electron microscopy (SEM) and 3D optical profilometry. The chemical interactions and molecular ordering were studied through Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) to understand hydrogen bonding and crystallinity modifications within the starch/PVA-PEG/TE matrix. Additionally, thermogravimetric analysis (TGA) was utilized to determine thermal stability and degradation behaviour under heat, key attributes concerning processing and usability. The functional performance was evaluated through standard antioxidant assay, such as DPPH radical scavenging, to measure oxidative protection capabilities along with migration studies. The cytocompatibility of the developed films were assessed through fibroblast (L929) cell viability tests to confirm the safety. Further, the antibacterial activity was also conducted against common foodborne pathogens, including *Escherichia coli* and *Staphylococcus aureus*, using colony forming unit methodology. To demonstrate practical applicability, the films was utilized for packaging of fresh-cut apples, which are susceptible to enzymatic browning, moisture loss, weight loss, and pH of the fruit.

2. Experimental section

2.1. Materials

Potato starch (ST) was purchased from Central Drug House Limited (India). Kollicoat® IR (PVA-PEG copolymer) and DPPH were obtained from Sigma-Aldrich. All other reagents used were of analytical grade. L929 fibroblast cells were obtained from NCCS, Pune, India. The two bacterial strains [*Escherichia coli* (MTCC 43) and *Staphylococcus aureus* (MTCC 96)] were procured from MTCC, India.

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Preparation of green tea extract (TE)

The green tea was collected from the institutional tea processing facility. The green tea extract (TE) was prepared by adding 5 g of green tea to 500 mL of distilled water and heat at 80 °C for 20 min with continuous stirring. The obtained solution was then filtered to remove any residues. The filtrate was condensed using rotatory evaporator (RV10, IKA, Germany) at 40 °C followed by freeze-drying [13, 18]. The extract was removed with help of spatula and grinded to obtain the green tea extract powder. The total phenolic content (TPC) of TE was estimated *via* Folin-ciocalteu method and expressed as gallic acid amount in mg/gm dry weight of the TE [19]. The TPC of all the samples was assessed in three replicates, and the average value was reported.

2.2. Preparation of Starch/PVA-PEG/Green tea extract films (STKTE films)

The different film forming solutions were prepared by blending ST and PVA-PEG co-polymer (KIR) as the primary film base using solvent casting method [18]. Briefly, Starch 6% (w/v) and PVA-PEG co-polymer 11 % (w/v) were dissolved in distilled water with continuous stirring at 60°C, in two separate beakers. Once completely dissolve, the solutions were combined in 1:1 ratio (v/v) and stirred for other 30 min. The tea extract (TE) at different concentrations (0.25, 0.5 and 1 % w/v) was added to primary film base to get three different films: (i) ST/KIR/TE 0.25 % (w/v) films (STKTE 0.25%); (ii) ST/KIR/TE 0.5 % (w/v) films (STKTE 0.5%); (iii) ST/KIR/TE 1 % (w/v) films (STKTE 1%). The obtained homogeneous solutions were casted on a flat surface with a digital adjustable applicator (VJ Instruments, India) to get films of uniform thickness. The films were dried at room temperature and stored till further use. The blank film STKB was also prepared, constituting of Starch and PVA-PEG co-polymer only. The thickness of films was determined using a digital micrometer and expressed in mm (millimetres).

2.3. Optimization of STKTE films

2.4.1. Film mechanical attributes

The tensile strength (TS) and elongation at break (EAB) of developed film samples were measured using a “tensile tester (SSIC-TTM-50 kgf-SC, SISCO, India)”. Prior to testing, the film samples were cut into 100 mm*20 mm strips and tested at a specific force rate.

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2.4.2. Water Contact Angle (WCA)

The WCA was measured using a "DMe-211 Plus contact angle meter (Kyowa, Japan)" following the sessile drop method[20]**.** Before experiment, the film samples (20 × 20 mm) were placed flat on the sample stage, and a droplet (2 ± 0.1 µL) of distilled water was deposited carefully on the film sample. The contact angle was recorded immediately after deposition followed by capturing of images and analyzed using FAMAS software.

2.4. Solid state characterization and barrier property analysis of STKB and STKTE 0.5% Films

2.4.1. Scanning Electron Microscopy (SEM)

The surface morphologies of STKB and STKTE 0.5% films were examined by "Scanning Electron microscope (SEM, Hitachi S-3400 N, 15 kV)". The film samples (10 × 10 mm) were fixed on metal stubs with help of adhesive carbon tape and sprayed with gold to ensure conductivity and clear imaging [20, 21].

2.4.2. 3D Optical profilometry

The topology and roughness of the STKB and STKTE 0.5% film samples were examined using an "optical profilometer (Contour GT-K, Bruker AXS, USA)" operated in confocal mode [22].

2.4.3. Colour and UV-shielding analysis

The UV–Visible spectrophotometer "(GENESYS™ 180, Thermo Scientific, USA)", was used to determine the transmittance of the STKB and STKTE 0.5% film samples. The transmission spectrum was recorded in the range between 200-800 nm[23]. The Color parameters L^* (lightness), a^*(red/green), b^*(Yellow/blue) and ΔE (Tota color difference) of the films were evaluated using a color Reader (CR6, China).

2.4.4. Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The possible molecular interaction between ST, PVA-PEG (KIR), TE, STKB and STKTE 0.5% films was studied by using an "infrared spectrophotometer (Agilent Technologies, USA)". The spectra were recorded between wavenumber ranging from 500–4000 cm^{-1} [24]**.**

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2.4.5. X-ray Diffraction (XRD)

The crystallinity of ST, PVA-PEG (KIR), TE, STKB and STKTE 0.5% films was examined with a "X-ray diffractometer (Malvern Panalytical diffractometer, UK)". The diffraction patterns were recorded over a 2θ range of 5° to 40° [5].

2.4.6. Thermogravimetric Analysis (TGA)

The thermal properties of ST, PVA-PEG, TE and STKB and STKTE 0.5% films were analyzed by a "Thermogravimetric analyzer, TA Instruments Discovery Series TGA5500 (Waters, USA)". Concisely, small amount of sample was placed into pans and heated from 25°C to 550°C at a rate of 20°C/min under nitrogen flow and % weight was recorded[25].

2.4.7. Opacity, Moisture Content, and Barrier properties of STKB and STKTE 0.5% films

The opacity of STKB and STKTE 0.5% films samples were analysed by recording absorbance using an "UV–visible spectrophotometer (GENESYS™ 180 UV-spectrophotometer, Thermo Scientific, USA)" at a specific wavelength of 500 nm [26]. The opacity was deduced using following equation:

$$\text{Opacity of films} = A_{500} * X \quad \text{.....equation 1}$$

where A_{500} is absorbance of film samples and X is thickness of film (mm).

The % moisture content of the STKB and STKTE 0.5% films was evaluated *via "*UniBloc moisture analyzer (MOC 63u, Shimadzu, Japan)". The water vapour permeability (WVP) of the film samples was tested by following ASTM E96 standard using payne permeability cups (Raj Make, India) [27]. Initially, the test cups were filled with 3 mL of distilled water, sealed with films and placed in vacuum desiccator for 24 h. The change in weight (g) of the test cups was recorded and WVP was calculated.

$$\text{Water vapor permeability of films} = \frac{\Delta W \times X}{A \times t \times \Delta P} \quad \text{...equation 2}$$

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Where, ΔW represents the test cup weight change (g), x is film thickness (mm), A corresponds to the film area (m²), t is the time period (s), and ΔP is the water vapor pressure difference (Pa).

An indirect method was used to analyse the oxygen transmission across the film[28]. Briefly, the centrifuge tubes containing 3 g of deoxidizer (iron powder), were sealed with films and placed at 25 °C and weighed after 48 hrs. The oxygen permeability (OP) of films was determined using following equation:

$$\text{Oxygen Permeability } (10^{-6}\text{g.mm.m}^{-2}.\text{s}^{-1}) = (\Delta\text{m} \times \text{d})/(\text{A} \times \text{t}) \quad \text{…equation 3}$$

where Δm is the mass change (g) of the tube, d is the film thickness (mm), t is the time (s) and A denotes the permeation area (m^2).

2.5. Determination of antioxidant activity of STKB and STKTE 0.5% films

The antioxidant activity of the STKB and STKTE 0.5% films was estimated using 2,2-diphenly-1-picrylhydrazyl (DPPH) assay [13, 21]. Briefly, 1 mL of various film concentrations (125, 250, 500, 750, and 1000 µg/mL) was added to 2 mL of DPPH solution, vortexed, and incubated in the dark for 30 min. The absorbance at 517 nm was determined using a "UV-spectrophotometer (GENESYS™ 180, Thermo Scientific, USA)" and % radical scavenging activity of films were deduced using the equation:

$$\%\ \text{scavenging activity of films} = \left(\frac{A_0 - A_1}{A_0}\right) * 100\% \quad \text{……..…equation 4}$$

Where, A_0 and A_1 denotes absorbance of the blank DPPH solutions and absorbance of solution with film sample, respectively.

2.6. Migration study of tea extract in STKTE 0.5% films

The migration behaviour of the TE incorporated in STKTE 0.5% film, was estimated using total immersion method with three different simulants (simulant A-water, simulant B- 3 % acetic acid and 95% ethanol)[29]. In short, film samples (1×3 cm) were immersed in 5 ml of simulant and incubated in dark at 40^0C for a period of 10 days. After incubation, the absorbance of the samples at 268 nm was recorded and migrated TE content was deduced by calibration curve of TE. Moreover, the antioxidant activity of the solution obtained after the migration test was also estimated using DPPH assay.

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2.7. Biocompatibility of STKB and STKTE 0.5% films

The fibroblast murine cells (L929 cells) was utilized to assess the biocompatibility of STKB and STKTE 0.5% films [21, 30]. Briefly, the L929 cells were seeded and treated with different film concentrations (5, 25, 50, 75 and 100 µg/mL) for 24 h at 37^0C. The MTT assay was used to assess the effect of films on L929 cells and % cell viability was calculated using the following equation:

$$\%\ \text{Cell viability of fibroblast cells} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) * 100 \quad \text{..... equation 5}$$

In addition, the cells were subjected to staining using the "Live/Dead™ Cell Imaging Kit (Invitrogen, Thermo Fisher Scientific)" after treatment with the films. The images of fibroblast cells were captured using the "ZOE™ Fluorescent Cell Imager (Bio-Rad)" to determine live and dead cells [31].

2.8. Antibacterial assessment of STKB and STKTE 0.5% films

The antibacterial efficacy of STKB and STKTE 0.5% films was assessed against two bacterial strains *viz. E. coli* (gram-negative) and *S. aureus* (gram-positive) [28]. Prior to experiment, the film samples were sterilized *via* UV light treatment for 30 min. Further, sterilized film samples (20 mg/mL) were dissolved in 25 mL of liquid medium inoculated with either *E. coli* (Luria broth) or *S. aureus* (Nutrient broth) and incubated for 12 h (at 37°C) with continuous agitation. The OD of samples were observed at specific intervals (1, 2, 4, 6, 8, 10 and 12h) and after incubation the diluted (6-fold) bacterial suspension (100 µL) was uniformly spread over the sterile agar plates. The number of colonies were counted with help of Handheld Digital Colony Counter (HIMEDIA). The bacterial suspension without film sample considered as control. The number of colonies counted were expressed in CFU/mL by using following equation:

$$\text{Colony Forming units per mL of film samples} = \frac{(\text{No. of colonies} \times \text{Dilution factor})}{\text{Volume of culture plated}} \quad \text{...equation 6}$$

2.9. Application in fresh cut apple preservation

Apple preservation was assessed using STKB and STKTE 0.5% films to estimate their potential in maintaining shelf life and quality of apple during storage. The fresh apples

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were procured from local market at Palampur (H.P), washed thoroughly and cut into cubed shaped pieces (2×2 cm). The apple cubes were divided into four groups (in triplicates with 3 apple cubes in each replicate) *viz.* Group 1: uncovered (control); Group 2: covered with conventional polyethylene packaging (PE); Group 3: covered with STKB film and Group 4: covered with STKTE 0.5% film. The fresh cut apples were stored at room temperature for a period of 5 days. All the groups were analysed during storage period for their Weight loss (%), pH and visual appearance[32]. For visual appearance, the images were captured at regular time intervals. The % weight loss was calculated using equation:

$$\text{Weight loss (\%) of apple cubes} = \left(\frac{W_i - W_d}{W_i}\right) * 100 \quad \text{……equation 7}$$

Where, W_i and W_d is the initial weight and weight at the day of the apple cubes, respectively.

The color parameters (L*, a* and b* value) of the apple cubes were measured using colorimeter "(CR-6, 3nh Technology, China)", at different time points during storage. The browning index (BI) was evaluated to determine the browning degree of the apple cubes during preservation period [33]. The BI of apple cubes was calculated according to following equation:

$$\text{BI of fresh cut apple} = \frac{y - 0.31}{0.172} \times 100 \quad \text{……equation 8}$$

$$\text{where, } y = \frac{a + 1.75L}{5.645L + a - 3.02b}$$

2.10. Soil burial test assessment of STKB and STKTE 0.5% films

The STKB and STKTE 0.5% film's physical disintegration was estimated by soil burial test [34]. Briefly, the film samples STKB and STKTE 0.5% (2× 2 cm) were placed between the mesh layers and buried at a depth of 10 cm in the soil. The films were regularly monitored and photographed at specific time intervals for a period of 10 days. The residual area of the film samples was measured using ImageJ software and calculated using following equation:

$$\%\ \text{residual film area} = \left(\frac{\text{Residual area of the film}}{\text{Initial area of the film}}\right) * 100 \quad \text{…equation 9}$$

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2.11. Statistical analysis

The statistical analysis of the obtained results was performed using GraphPad Prism 10 (GraphPad Software Inc., CA, USA). The t-test and one-way or two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test was employed to compare differences between the groups with statistical significance considered at $p < 0.05$. All results were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Optimization and selection of tea extract incorporated ST/PVA-PEG copolymer/TE (STKTE) film

Starch-based packaging films have gained considerable interest as eco-friendly alternatives to petroleum-based plastics due to their biodegradability, renewability, and cost-effectiveness. However, films composed solely of starch often face several limitations, such as high brittleness, inadequate mechanical strength, and significant hydrophilic tendencies, which lead to increased moisture sensitivity and reduced water resistance [6, 22, 35-38]. These challenges limit their direct application in food packaging and other uses that require flexibility, durability, and moisture stability. To overcome these issues, a PVA-PEG copolymer was integrated into the starch matrix. This copolymer has inherent plasticizing properties thanks to its flexible ether linkages and hydroxyl groups, which enhance intermolecular hydrogen bonding and facilitate chain mobility [22, 39].

Further, TE was incorporated as a functional additive to impart additional active properties and reinforce the film structure. TE, rich in polyphenolic content (458.9 ± 0.5 mg GAE/g), can form strong interactions with polymeric chains through hydrogen bonding and hydrophobic interactions [19, 40-43]. The addition of such extracts impacts the films mechanical and barrier properties in multifaceted manner. Therefore, the ST/PVA-PEG copolymer films were optimized based on tensile strength, elongation at break and water contact angle with varying concentration of tea extract. The TE was incorporated in three different concentrations (w/v) into the polymeric solution which resulted in formation of three different types of films viz. STKTE 0.25 %, STKTE 0.5 %, and STKTE 1 % **(Figure 2)**.

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The blank film composed of only Starch and PVA-PEG copolymer demonstrated the TS of 2.2 ± 0.9 MPa, EAB of 1.7 ± 0.5 % and WCA of 58.6 ± 0.6°. The addition of TE in the polymeric matric impacts the mechanical and water contact angle in a bell-shaped manner [22, 24, 42]. Specifically, the film with lowest concentration of TE (STKTE 0.25 %) exhibited lowest TS (1.03 ± 0.4 MPa), EAB (2.2 ± 0.5 %), and WCA (60.9 ± 5.4°). Further, a significant enhancement in the mechanical as well as WCA of film was observed in film with 0.5% of TE (STKTE 0.5 %) which showed TS of 4.7 ± 0.3 MPa, EAB (3.6 ± 1.5 %), and WCA (70.7 ± 0.3°), indicating enhanced intermolecular interaction between TE and film components [22]. However, further increase in TE amount (STKTE 1 %), the mechanical attributes and WCA decreases, might be due to saturation and agglomeration. Conclusively, STKTE 0.5 % film demonstrated enhanced mechanical and WCA parameters, therefore selected for further experiments.

< Insert Figure 2>

3.2. Characterization of developed ST/PVA-PEG copolymer/TE (STKTE) film

3.2.1. Surface and morphological analysis of STKB and STKTE 0.5% film

SEM and 3D profilometry analysis was conducted to observe the surface morphology and microstructural variations before and after incorporation of TE in the film **(Figure 3)**. The SEM analysis reveals that the morphology shifts from larger, irregular domains in the pure STKB film to smaller, more uniformly distributed domains in the STKTE 0.5% film, implying improved dispersion or interaction at the microscopic level. Further, the optical profilometry data demonstrated slight increase in the average peak height (R_{pm}: 6.5 ± 2.4 µm) and R_z (roughness of film) value (37.9 ± 5.4 µm) in STKTE 0.5% film in comparison to STKB film (R_{pm}: 4.3 ± 1.2 µm and R_z: 35.7 ± 3.03 µm). The R_{pm} and R_z values provide essential information on surface roughness, which directly influences the mechanical integrity, wettability, and overall uniformity of food packaging films. The lower values of R_z indicate a smooth and uniform film surface, which is generally important for ideal packaging film [44]. These combined changes of SEM and optical profilometry, suggests that TE modifies the microstructure of film, leading to enhanced surface texture and altered optical properties [6, 45].

< Insert Figure 3>

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3.2.2. Optical and barrier attribute analysis STKB and STKTE 0.5% film

Colour, opacity and light transmittance are crucial parameters for packaging film applications, as these impact on the product freshness and consumers perception. A higher opacity value indicates greater absorption of visible light by the film at a specific thickness, thereby reducing light transmission through the film. The addition of TE changes color of the film and also impacted the % transmittance and opacity of the film **(Figure 4)**. The colorimetric analysis revealed that the STKTE 0.5% sample exhibits a noticeable yellow coloration, with high b* value (42.90) and positive a* value (17.24), along with a lower lightness value (L* = 51.92) compared to the almost colorless STKB sample (L* = 87.89). The large color difference (ΔE = 51.16) between the two samples confirms the visible color change upon TE addition[6].

< Insert Figure 4>

The ultraviolet (UV) radiation significantly contributes to the deterioration of food quality; hence, packaging films should offer sufficient transparency while effectively blocking UV light to ensure product preservation. The UV-blocking capability of the films was assessed using a UV spectrophotometer [13]. The results revealed that STKTE 0.5% film completely absorbed the UV region light (UVA, UVB and UVC light) and demonstrated negligible transmittance across the film. This remarkable UV blocking ability of the STKTE 0.5% was due to the polyphenolic constituents present in the TE which effectively absorb ultraviolet radiation [40, 46]. The incorporation of 0.5% TE into STKB not only improves UV protection but also imparts a distinct yellow tint and reduces the lightness of the film. Similarly, the STKTE 0.5% film showed high opacity value 0.039± 0.00007 as compare to STKB film (0.028 ± 0.0002) **(Figure 5a)**. This confirms that in STKTE 0.5% film, addition of tea extract (TE) improved the light absorbing property of the films.

Further, the ability of packaging films to restrict the transmission of water and gases is a critical determinant of their effectiveness in food preservation. Among these, water barrier properties are particularly important, as they help maintain the moisture balance, texture, and overall stability of food products during storage [28, 47]. These characteristics are commonly evaluated through measurements of moisture content, WVP and oxygen permeability. The WVP of a film is largely governed by its thickness,

degree of crosslinking, and polymer chain mobility. Additionally, oxygen exposure can accelerate undesirable processes such as lipid oxidation, discoloration, and microbial growth, ultimately leading to food spoilage [48]. Collectively, these factors are essential for preserving the freshness, sensory quality, and nutritional value of foods while extending their shelf life. Therefore, the developed films were assessed for these parameters to comprehensively evaluate their barrier performance.

The results revealed that the moisture content % of the STKTE 0.5% film (17.16 ± 0.44 %) significantly reduced compared to STKB film (22.28 ± 1.94 %) **(Figure 5b)**. The decrease in the moisture content % of the tea extract loaded (STKTE 0.5%) film, due to the inclusion of the hydrophobic constituents of TE, which lowers the water adsorption capability of the film. Similar results were reported by wen et al., in their study on pH-sensitive Poly (vinyl alcohol) films incorporated with green tea extract [40]. Similarly, notable decrease in WTR and OTR of the STKTE 0.5% was observed **(Figure 5c and d)**. Specifically, the STKTE 0.5% film exhibited significantly lower WVP ($1.68 \pm 0.07*10^{-7}$.g.mm/sec.m^2.Pa) than STKB film ($2.17\pm0.12*10^{-7}$.g.mm/sec.m^2.Pa). The WVP of the STKTE 0.5% film was decreased due to addition of TE containing bulky aromatic skeleton and can obstruct the inner network of the tea extract loaded film (STKTE 0.5%), corresponds to lower vapor affinity of the films [40, 42]. The oxygen permeability of the films followed the similar trend as the WTR. The STKTE 0.5% film exhibited significant decrease ($5.97 \pm 0.63*10^{-6}$.g.mm/m^2.s) in the oxygen permeability as compared to STKB film (13.07 ± 1.1810^{-6}.g.mm/m^2.s). The TE present in the STKTE 0.5% film, acted as a barrier that successfully inhibited the diffusion of the oxygen molecules, which corresponds to lower oxygen permeability. Also, the crosslinking of the film materials, reduced the free space present in the film, resulted in low OP values[14]. These findings indicated that the addition of 0.5% TE not only increases film opacity but also enhances its moisture resistance and gas barrier performance, making the modified film potentially more suitable for packaging applications.

< Insert Figure 5>

3.2.3. Molecular, solid state and thermal analysis of STKB and STKTE 0.5% films

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The FTIR spectra of the film and film components were analysed to understand the intermolecular interaction between the components of films **(Figure 6a)**. The FTIR spectra of starch showed band at 3399 cm^{-1} and 2929 cm^{-1} corresponding to the symmetric and asymmetric stretching vibrations of O-H and C-H groups. The absorption peak at 991 cm^{-1} was attributed to the hydrogen bond formed by oxygen atom on the starch glycosidic bond [5, 23, 49]. The FTIR spectrum of the PVA-PEG copolymer (KIR) showed a broad and intense absorption band between 3600 and 3000 cm^{-1}, related to O–H stretching vibrations, indicative of strong hydrogen bonding interactions. Two distinct peaks at 2897.18 cm^{-1} and 1433.04 cm^{-1} were attributed to asymmetric CH_2 stretching and CH–O–H bending vibrations, contributed to its polymeric backbone structure. Additionally, two characteristic peaks at 1241.50 cm^{-1} and 1084.05 cm^{-1} corresponds to C–O–C stretching of the alkyl ether group and C–O stretching vibrations, respectively [17, 50]. The FTIR spectra of TE demonstrated the absorption at 1350 cm^{-1} and 1446 cm^{-1}, which attributed to C-H stretching and peak at 1647 cm^{-1} contributed to c=C stretching [40]. The FTIR spectra of STKB and STKTE 0.5% film exhibited the broadening and shifting of peaks of film components in the region between 3700 – 3000 cm^{-1} and 1500 – 1800 cm^{-1} demonstrating possible hydrogen bonding linkage between the film polymers.

The crystal structures of the ST, PVA-PEG copolymer (KIR), STKB, STKTE 0.5% were determined using XRD analysis **(Figure 6b).** The XRD pattern of ST exhibited high intensity peak at 17 and low intensity peaks at 15, 22.6 and 24.2, demonstrating its partial crystalline nature [5, 23]. On the other hand, the diffractogram of KIR attributed only one defused pattern at 19.3 and 22 (2θ), typical of semi-crystalline polymeric structures [51]. Similarly, the XRD spectra of TE demonstrated its partial amorphous nature due to the presence of numerous components including fibres, tea polyphenols and catechins. Further, the XRD diffractogram of film samples (STKB and STKTE 0.5%) demonstrated diffused and halo spectra, revealing amorphization of the film components. This transition toward an amorphous structure reflects strong interfacial interactions between the components, which may enhance material uniformity and performance.

The thermal stability of the developed films was analyzed by thermogravimetric analysis. TGA thermogram of all samples demonstrated multi-step degradation,

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typically involving initial moisture loss followed by the decomposition of the organic matrix **(Figure 6c)**. Specifically, degradation of ST initiated at 246.1 °C to 378.5 °C and 378.5 °C -548.7 °C corresponding to 52.1 % and 27.21 % weight loss with residual weight of 7.19 %, respectively [25, 52]. The TGA profile of PVA–PEG copolymer (KIR) exhibited a distinct two-step degradation pattern [53]. The initial stage showed a minor weight loss of approximately 1.3% below 150 °C, corresponding to the evaporation of physically adsorbed and bound water molecules. The primary decomposition phase occurred between 163 °C and 432.6°C, resulting in a weight loss of about 81.33%, which can be attributed to the degradation of organic constituents and partial cleavage of the polymer backbone. Subsequent degradation events between 432.6°-483.2 °C and 483.2 °C-550 °C contributed additional weight losses of 6.17% and 10.6%, respectively. The thermogram of TE displayed a broad degradation band beginning at around 150 °C, with a peak near 200 °C till 392 °C corresponding to 95 % weight loss. This transition is attributed to the thermal decomposition of glycosylated catechins, where the attached sugars undergo caramelization upon heating. Additionally, the partial degradation of catechins, leading to the formation of gallic acid and subsequent polymerization of phenolic compounds, contributes to this thermal event. The degradation extended over a wide temperature range, with a final stage observed beyond 340 °C, corresponding to the thermal decomposition of cellulose components present in the TE[40].

The thermal stability of the film samples improved upon the incorporation of TE in film components, as observed in the STKTE 0.5% curves, which exhibit delayed onset of degradation from 200°C (in TE) to 229 °C. Moreover, the TGA curve of STKTE 0.5% curves demonstrated gradual increase in weight loss in despite of sharp increase in degradation as observed in TE curve. This enhancement can be attributed to the reinforcing effect and thermal barrier properties imparted by the additives.

Overall, the combined FTIR, XRD, and TGA results confirm the successful integration of PVA-PEG copolymer (KIR) and TE into the ST matrix, leading to chemical interactions, reduced crystallinity, and improved thermal stability. These modifications suggest that the composite film enhanced the structural homogeneity and thermal resistance compared to the unmodified samples.

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3.3. Antioxidant activity and biocompatibility of STKB and STKTE 0.5% films

The antioxidant ability (free radical scavenging activity) is crucial for food packaging films, as free radicals generated in food can cause oxidation and spoilage of food. The DPPH radical scavenging activity is important to estimate antioxidant property of film samples. The results demonstrated that DPPH scavenging activity of STKTE 0.5% film exhibited concentration dependent increase as compared to STKB film, with 84% scavenging at the highest concentration (1000 µg/mL) **(Figure 7a).** However, STKB film showed only 7 % scavenging at highest concentration (1000 µg/mL). The addition of TE improved the antioxidant potential of the film (STKTE 0.5%), due to the presence of phenolic compounds present in TE. The TE components are known to disrupt chain oxidation reaction, releasing hydrogen atom and acts as a receptor for free radicals [13, 27, 54]. This suggested that the addition of TE significantly influenced the antioxidant activity of the films.

To determine the cytocompatibility of the developed films (STKB and STKTE 0.5%), *in vitro* biocompatibility was performed using L929 mouse fibroblast cell line. The film samples were incubated with fibroblast cells followed by MTT assay to determine cell viability. In case of both films (STKB and STKTE 0.5%) the cell viability observed was more than 90%, suggesting that films are biocompatible and non- toxic to cells **(Figure 7b)**. Despite the incorporation of tea extract (TE), STKTE 0.5% film sample maintained high cell compatibility, demonstrating that neither its concentration nor its incorporation method induced any adverse cellular response [21, 55]. The excellent biocompatibility of these films supports their potential role for interaction with biological tissues extending their application beyond the food packaging.

<Insert Figure 7>

3.4. Tea extract migration analysis

In general, the release/migration of active component from the film is critical for providing effective functional attributes to film. This migration also depends upon the type of food preserved in the packaging material and its rate depends upon compatibility between film polymer, food simulant and active component [37]. Therefore,

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the migration of TE from STKTE 0.5% films was analyzed in three different food simulants (3% acetic acid, 95% ethanol and water) (Supplementary table S1). The results of migration study revealed that film in 3% acetic acid (79.7 ± 2.0 %) and water simulant (77.3 ± 2.7 %) showed maximum release/migration of TE from film to solution. However, the film incubated in 95% ethanol demonstrated 51.5 ± 4.4 % migration of TE, possibly because of the limited solubility of starch in ethanol. Further, the DPPH assay of the simulant solution also confirmed the effective migration and retention of antioxidant activity of films (Supplementary figure S1). Specifically, antioxidant assay results revealed that film in 3% acetic acid (80.1 ± 0.2 %) and water (72.7 ± 0.2 %) demonstrated highest and equivalent DPPH scavenging activity to that of STKTE 0.25% film without simulant treatment in comparison to film in 95% ethanol simulant (60.1 ± 0.2 %). The higher antioxidant activity in acetic acid and water, may be because of the higher solubility of film in these simulants in comparison to ethanol. The starch alone is usually less soluble in water, but the incorporation of the PVA-PEG copolymer increased its solubility by forming hydroxyl groups and allows the antioxidant compounds to release more effectively from film samples[56]. The similar results were obtained in a study conducted on mixing the potato starch with PVA, which results in formation of hydrophilic films and increase the solubility of films in water, mainly because of increase in number of -OH groups [57].

3.5. Antibacterial efficiency of STKB and STKTE 0.5% films

The antibacterial properties of developed films can inhibit the growth of potential foodborne pathogen, thereby limit the foodborne illnesses and prolong the food shelf-life. The antibacterial efficiency of developed films was tested against two bacterial strains: *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria and determined by colony Forming Unit (CFU/mL) method **(Figure 8)**. The control and STKB film exhibited intense bacterial growth in comparison to STKTE 0.5% against both the bacterial strains (*S. aureus* and *E. coli*). Specifically, the STKTE 0.5% film showed, 7.9×10^6 CFU/mL against *S. aureus* and 3.3×10^6 CFU/mL against *E. coli*, which indicates that STKTE 0.5% film exhibited significant antibacterial effect against *E. coli* as compared to *S. aureus* **(Figure 8)**. The results demonstrated that developed STKTE 0.5% film showed promising inhibition on growth of both the bacterial strains in comparison to STKB film. The antibacterial property of the STKTE 0.5% film can be

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attributed to incorporation of the TE, which contain polyphenols, and have the potential to inhibit the growth of wide variety of bacteria especially gram-positive and gram-negative species [13]. The observed results are supported by the findings of Lie et al., reported the significant inhibition of *E. coli* than *S. aureus* at equivalent concentration of TE [24].

<Insert Figure 8>

3.6. Fresh cut apple preservation

The ability of the STKB and STKTE 0.5% films to preserve fresh cut apple was examined by monitoring several quality parameters *viz*., visual appearance, color parameters, % weight loss, pH and BI[32]**.** In terms of visual appearance, the fruits packed in STKTE 0.5% film effectively maintained their appearance till the 5th day of experiment followed by STKB, PE and control groups **(Figure 9a)**. Moreover, the color parameters ($L^*a^*b^*$ values) of the apple cubes were also in corroboration with the visual appearance, confirming the color changes during storage **(Figure 9b)**. Further, the browning index of apple was also evaluated and results demonstrated browning index of apple cubes was highest in control as the fruit cubes were not protected. However, in case of covered fruit cubes the browning index decreased from PE > STKB >STKTE 0.5% **(Figure 9c)**. The possible cause of fruit browning could be the polyphenols oxidation to produce quinones which reacts to generate brown/ black pigments [33, 58].

The weight loss assessment of stored apple cubes was also estimated for a period of 5 days and it was observed that the weight loss was maximum (57.1 ± 10.4) in the control group (uncovered apple cubes). In contrast the covered apple cubes exhibit minimum weight loss starting from PE (52.0 ± 10.7) followed by STKB (30.2 ± 6.6) and then STKTE 0.5% (28.6 ± 6.4**) (Figure 9d)**. The weight loss observed in all the groups, is likely to associated with the rapid increase in respiration just after the cutting of fruit. Moreover, since moisture loss is directly associated with film permeability, the weight loss results can be interpreted on basis of WVP values [58, 59]. The film with lower WVP (STKTE 0.5%) exhibited least weight loss, whereas STKB film with higher WVP showed greater weight loss than STKTE 0.5%.

The pH serves as a key indicator of fruit freshness and spoilage. The decline in apple pH during storage is primarily attributed to the accumulation of acidic metabolites,

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enzymatic breakdown of cell wall components, and potential microbial fermentation. Together, these processes elevate the fruit's overall acidity, signalling progressive deterioration in quality [32]. Therefore, the pH of apple was estimated at the end of the experiment, revealing that the pH in control group found to be the lowest (2.47 ± 0.03) as compare to other groups. The STKTE 0.5% group found best to maintain the pH (2.63 ± 0.02) of the fruits **(Figure 9d**) in comparison to blank (2.53 ± 0.05) and commercial packaging film (2.48 ± 0.04).

<Insert Figure 9>

3.7. Soil burial test assessment

The physical disintegration assessment of the STKB and STKTE 0.5% films was carried out using soil burial method for a period of 10 days [34]. The STKTE 0.5% film showed significant reduction in film area with residual area of 0.6 ± 0.6% as compare to STKB film (3.4 ± 2.0 %), confirming its high vulnerability towards microbial degradation and breakdown in environmental conditions **(Figure 10).** Overall, both the films STKB, STKTE 0.5% showed the potential to serve as sustainable alternatives for conventional food packaging materials.

<Insert Figure 10>

4. Conclusion

In nutshell, the study presents the successful development of biodegradable and functional films based on starch and PVA-PEG (Kollicoat IR) copolymer incorporated with green tea extract (TE) as a natural bioactive additive. The incorporation of 0.5% w/v TE markedly enhanced the mechanical strength, water contact angle and barrier properties of the films, demonstrating superior performance compared to the blank STKB film. The structural and thermal analyses (SEM, 3D profilometry, FTIR, XRD, and TGA) confirmed improved film uniformity, strong intermolecular interactions, and enhanced thermal stability upon TE addition. The developed films exhibited enhanced antioxidant and antibacterial activities, effectively inhibiting *E. coli* and *S. aureus*, while maintaining cytocompatibility in L929 fibroblast cells. The application of the films for packaging fresh-cut apples significantly reduced browning, weight loss, and pH decline, indicating extended shelf life and improved preservation quality. Moreover,

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the films displayed rapid physical disintegration within 10 days under soil burial conditions, confirming their environmental sustainability. Overall, the developed ST/PVA-PEG/TE films present a promising green alternative to conventional plastic packaging, combining biodegradability, bioactivity, and functional performance suitable for food preservation and sustainable packaging applications.

Supplementary Information

Supplementary information includes table of percent migration of tea extract from STKTE 0.5% film and figure of DPPH assay of film samples after migration study conducted for 10 days in different food simulants (3% Acetic acid, water and 95% ethanol).

Data Availability Statement

Data supporting the findings of this study are available within the manuscript.

CRediT authorship contribution statement

Neha Rana: Writing - original draft, Methodology, Investigation, Data curation, Formal analysis. **Ruchika**: Methodology, Investigation, Writing - original draft, Formal analysis. **Ankit Saneja**: Writing - review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

Figure	Legend
Figure 1	(a) Schematic illustration of development of starch/PVA-PEG copolymer packaging film reinforced with green tea extract and its assessment for mechanical, barrier and solid-state characterization. (b) The developed film was further analysed for functional properties including antioxidant and antibacterial, postharvest apple preservation, biocompatibility and biodegradability.
Figure 2	Mechanical and surface properties of STKB and STKTE films with varying concentrations of green tea extract (TE). (a) Representative stress–strain curves demonstrate the mechanical behavior of the samples under tensile loading. (b) Tensile strength, (c) elongation at break, and (d) water contact angle measured for STKB and STKTE films containing 0.25%, 0.5%, and 1% w/v of TE. Data are presented as mean ± standard deviation (n = 3). Statistically significant differences are indicated by different letters above the bars: "a" denotes a significant difference from STKB, "b" from STKTE 0.25%, and "c" from STKTE 0.5%. In d) graph representative images of water droplets used for contact angle measurements are shown above each corresponding bar.
Figure 3	Optical appearance, surface morphology, and topography of STKB and STKTE 0.5% films. The STKB film (a) appears highly transparent in the photographic image, allowing clear visibility of printed text under it. The corresponding SEM image, reveals a relatively smooth surface with some dispersed features. Optical profilometry of the STKB film shows a moderately uniform surface with height variations ranging from –36.6857 µm to 5.83474 µm. In contrast, the STKTE 0.5% film (b) shows a distinct yellowish tint in the photographic image and reduced transparency. The SEM image at the same magnification

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	indicates a more heterogeneous surface with more irregular features, suggesting increased surface roughness. This is further confirmed by the optical profilometry data, which reveals a less uniform surface topology with height differences ranging from –35.9652 µm to 4.71027 µm.
Figure 4	Optical and colorimetric properties of STKB and STKTE 0.5% films. (a) UV–visible transmittance spectra, where STKTE 0.5% exhibits significantly reduced transmittance across the UV region (UV-C to UV-A), indicating enhanced UV- shielding compared to STKB. (b) The CIE Lab* color coordinates, with STKTE 0.5% shifting markedly toward the red-yellow quadrant, while STKB remains near the neutral centre. (c) photographic images of both films and their corresponding colorimetric values. STKTE 0.5% shows a much lower lightness (L*) and higher chromaticity (a*, b*), resulting in a notable color difference (ΔE = 51.16) relative to STKB.
Figure 5	The comparison of physical and barrier properties between STKB and STKTE 0.5% films. (a) Opacity of the films increased significantly with the addition of 0.5% STKTE, indicating higher light-blocking capability. (b) Moisture content was significantly reduced in the STKTE 0.5% films compared to STKB, suggesting improved water retention characteristics. (c) Water vapor permeability (WVP) decreased in STKTE 0.5% films, indicating enhanced barrier properties against moisture. (d) Oxygen permeability (OP) was also significantly lower in the STKTE 0.5% films, reflecting better resistance to gas transmission. Data are presented as mean ± standard deviation (n = 3), and bars with “a” indicate statistically significant differences ($p < 0.05$) from STKB film.
Figure 6	Solid state characterization of ST, KIR, TE, STKB, and STKTE 0.5% samples. (a) Fourier-transform infrared spectroscopy (FTIR) spectra showing characteristic functional groups and chemical interactions among the materials. (b) X-ray diffraction (XRD) patterns highlight the crystallinity and structural changes of the samples. (c)

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	Thermogravimetric analysis (TGA) curves displaying thermal stability and decomposition profiles as a function of temperature.
Figure 7	Antioxidant activity and biocompatibility of STKB and STKTE. (a) DPPH scavenging assay shows dose-dependent antioxidant activity for STKTE (0.5%), significantly higher than STKB at all concentrations ($p < 0.05$). (b-i) L929 cell viability graph and (ii) Fluorescence images demonstrate predominantly live cells (green) supporting the biocompatibility of both extracts. The Scale bar in the microscopic images is of 50 µm.
Figure 8	Antibacterial effects of STKB and STKTE 0.5% against *S. aureus* and *E. coli.* (a) Representative agar plates of *E. coli* demonstrate dose-dependent bacterial inhibition, with reduced colony formation in both strains and Growth curves (OD_{600}) demonstrated both treatments reduce bacterial growth over 12 h compared to control, with STKTE exhibiting stronger inhibition (b) Representative agar plates of *S. aureus* demonstrate dose-dependent bacterial inhibition, with reduced colony formation in both strains, particularly *E. coli*, at higher concentrations of STKTE and Growth curves (OD_{600}) over 12 h compared. (a $p < 0.05$ vs. control; b $p < 0.05$ vs. KIRB).
Figure 9	Effects of different treatments on the quality of fresh-cut apples over 5 days of storage. (a) Visual appearance shows that STKTE 0.5% best preserved color and texture, while the control showed the most browning. (b) Heatmaps of color values (L*, a*, b*) indicate that STKTE 0.5% retained lightness and color better than other treatments. (c) Browning index increased in all samples, but was lowest in STKTE 0.5%. (d) Weight loss was highest in the control and lowest in STKTE 0.5%, indicating improved moisture retention. (e) Graph depicting change in pH of fruit after the storage of 5 days. (a $p < 0.05$ vs. control; b $p < 0.05$ vs. KIRB).
Figure 10	Soil burial test assessment of STKB and STKTE 0.5% films over time. (a) Representative images showing the physical degradation of STKB and STKTE 0.5% films over a 10-day period. (b) Quantitative analysis of % residual area for STKB (green line) and STKTE 0.5% (blue line)

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films over time. Data represent mean ± standard deviation (n = 3). STKTE 0.5% films exhibited slightly faster degradation compared to STKB.

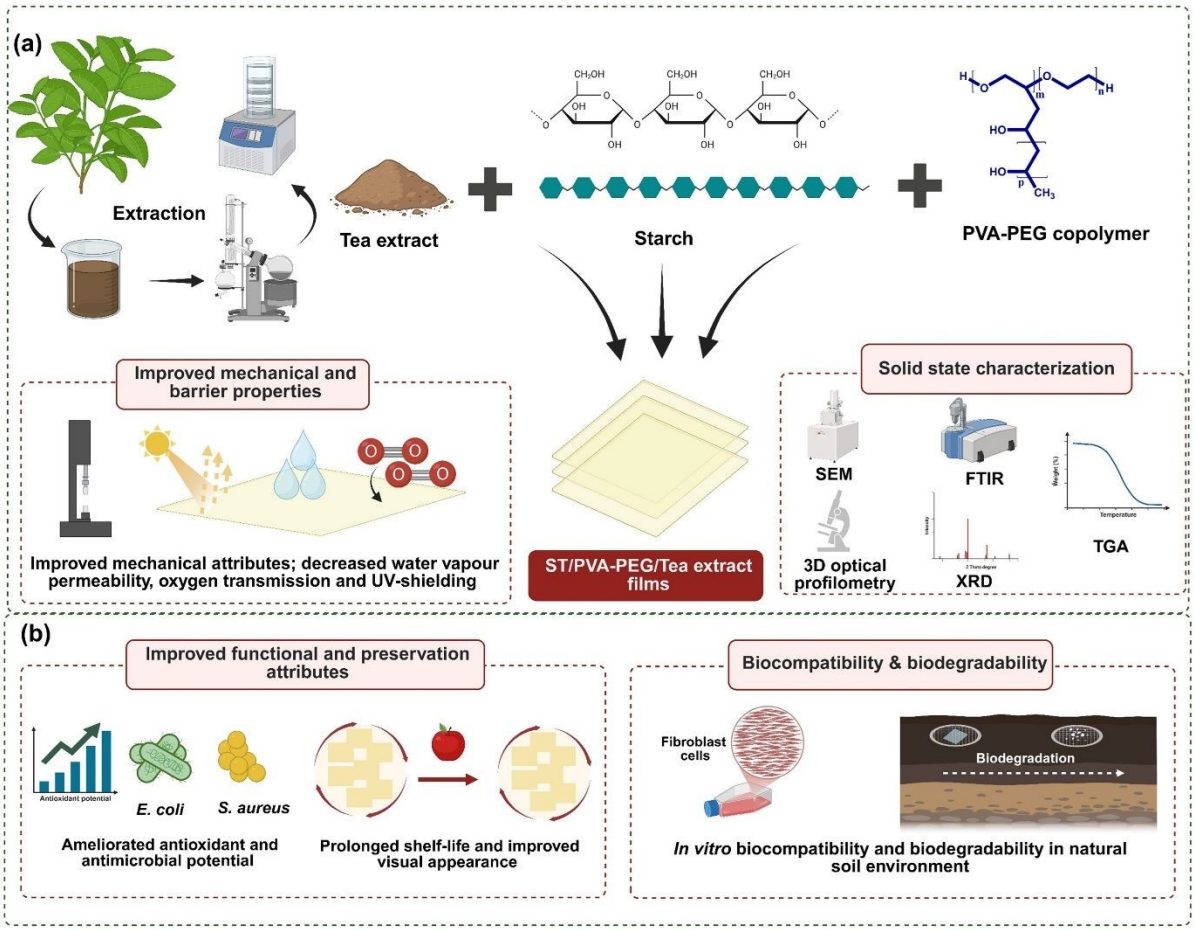

Figure 1

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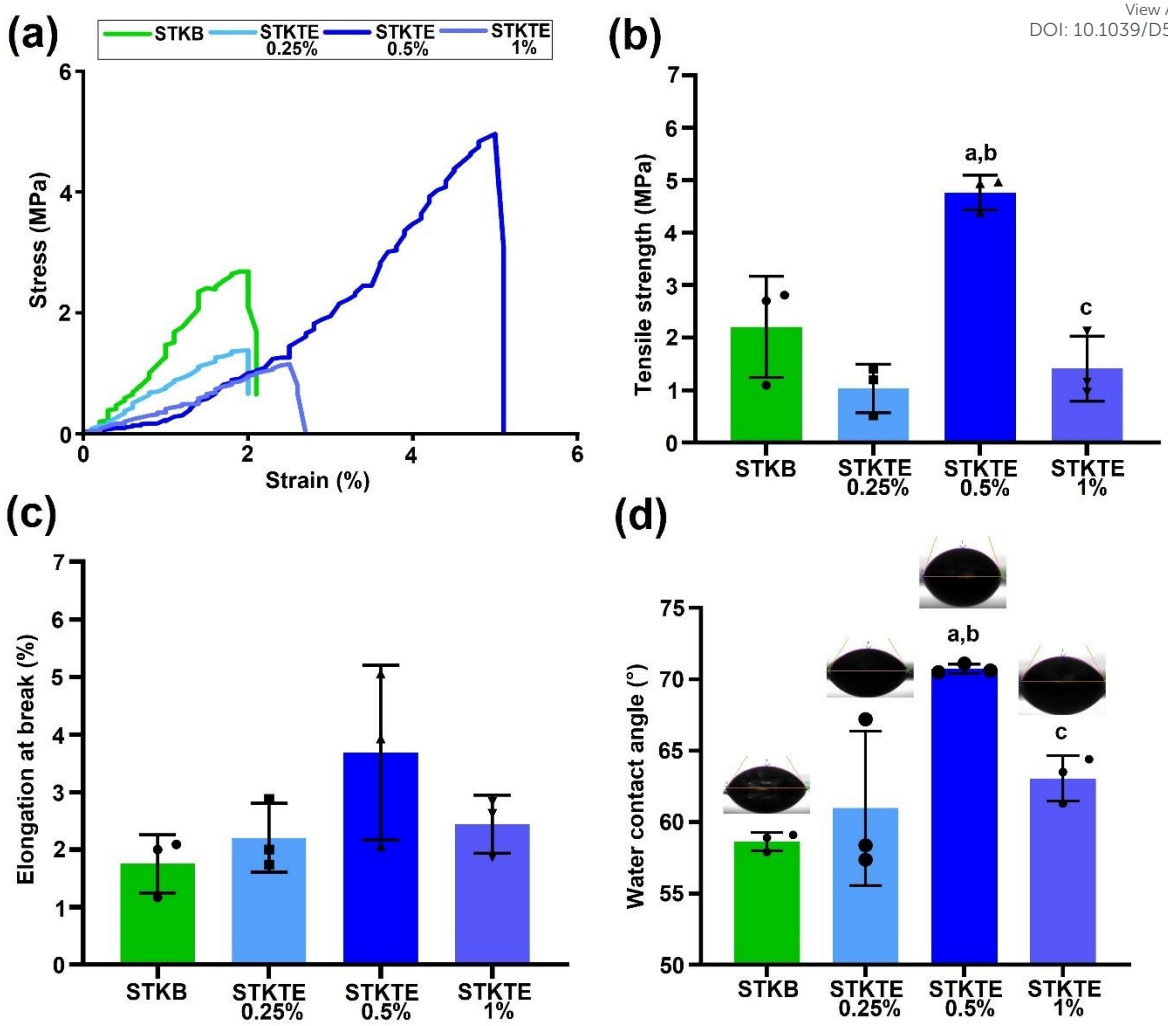


763

764 **Figure 2**

765

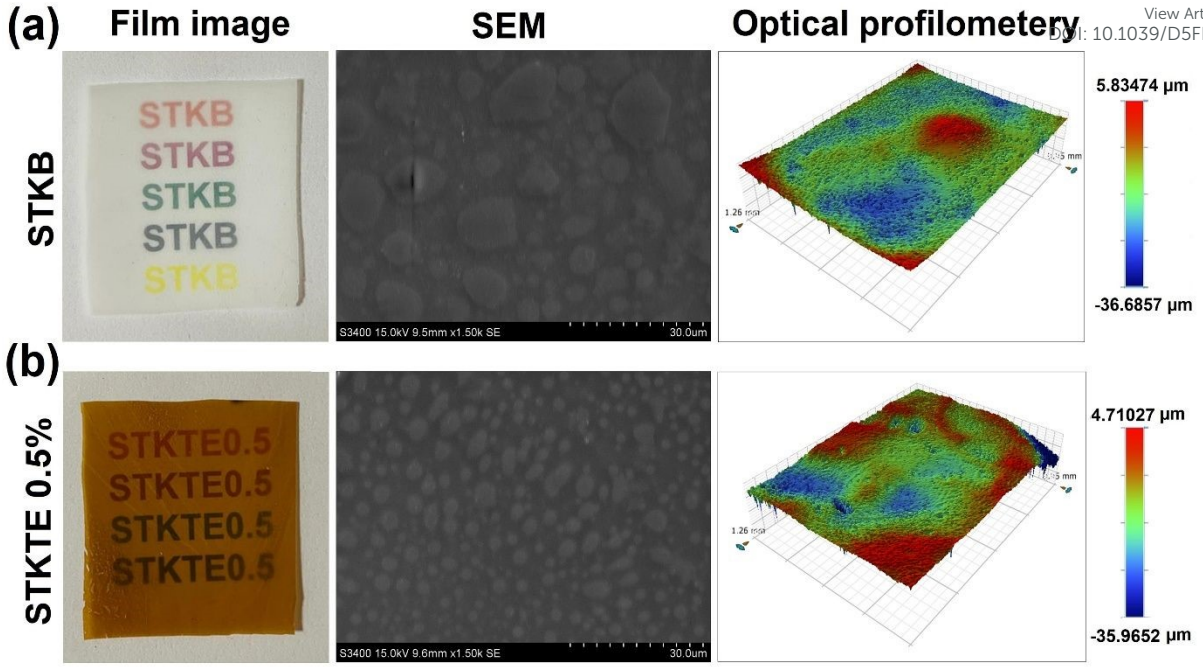
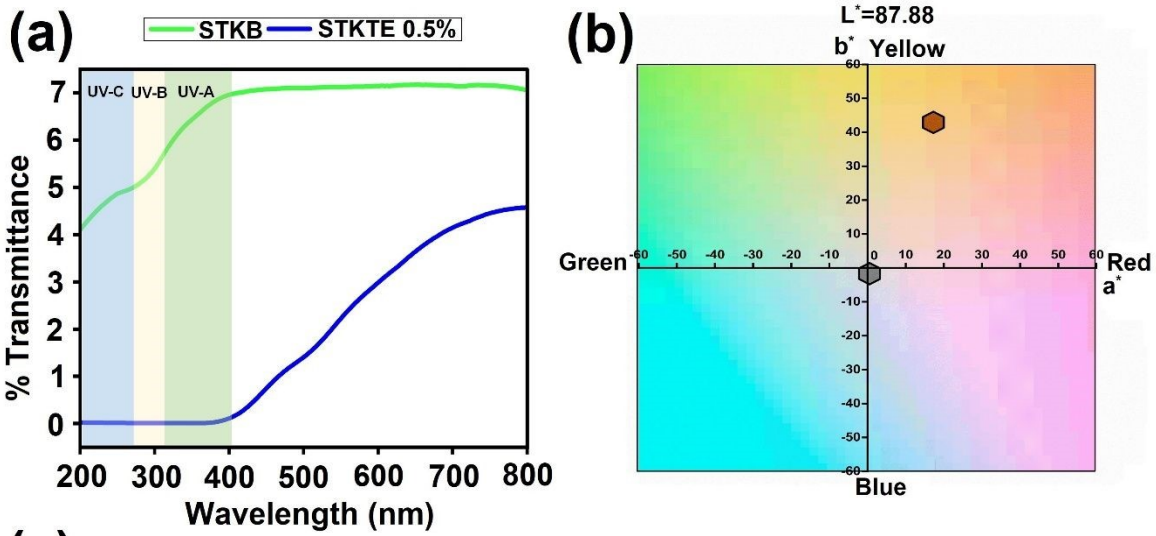


Figure 3



(c)

	L^*	a^*	b^*	ΔE
STKB	87.89±0.12	0.53±0.12	-1.72±0.05	-
STKTE 0.5%	51.92±0.35	17.24±0.14	42.90±0.28	51.16±0.25

Figure 4

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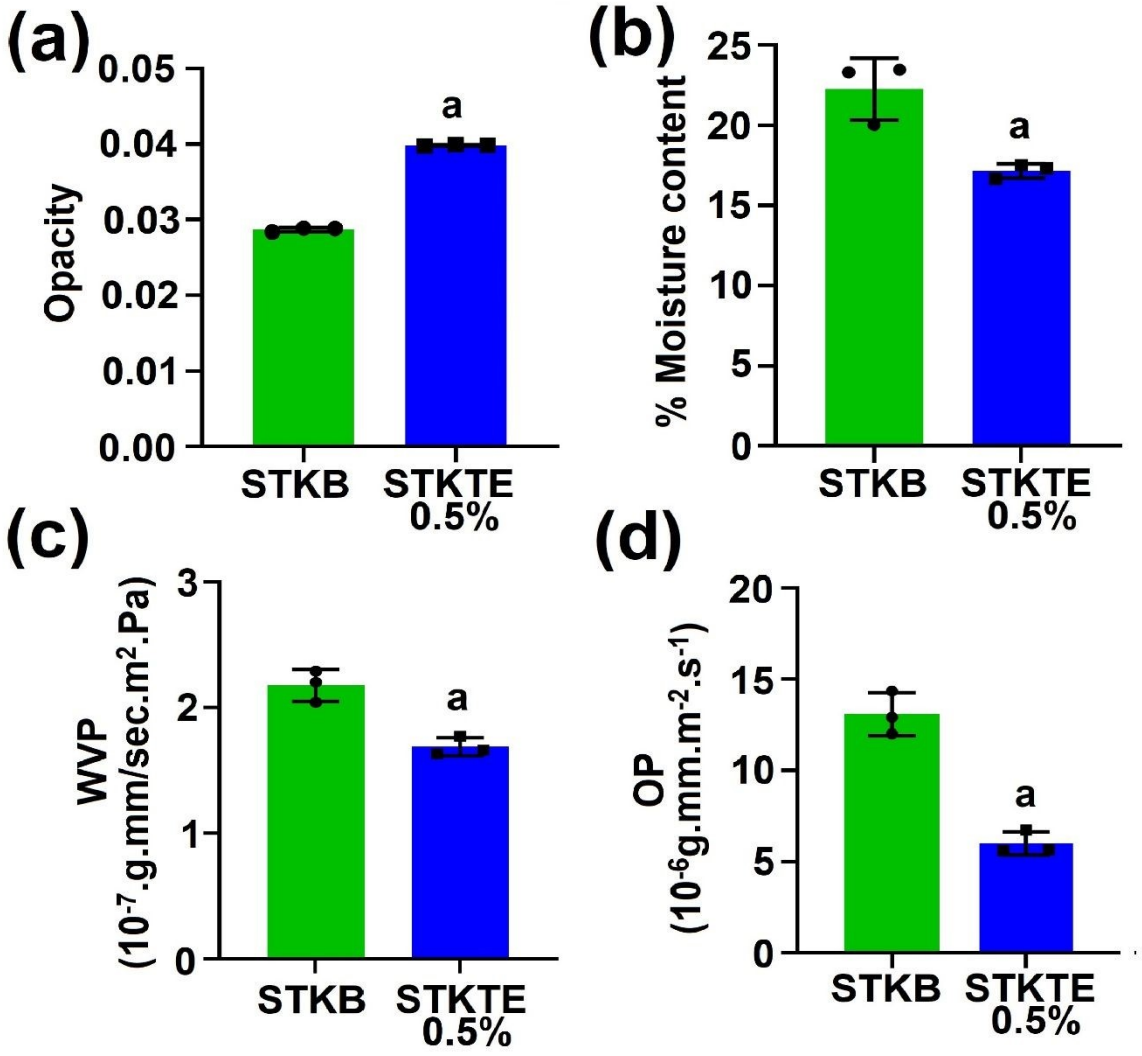

Figure 5

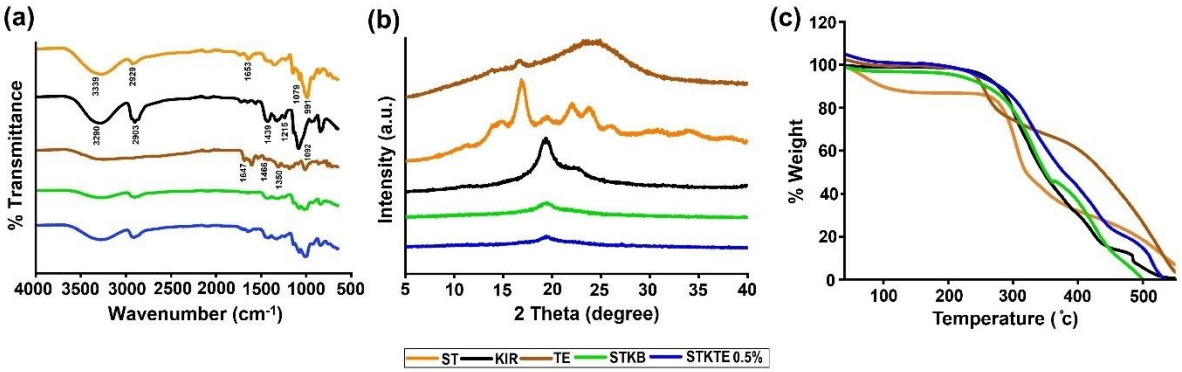

Figure 6

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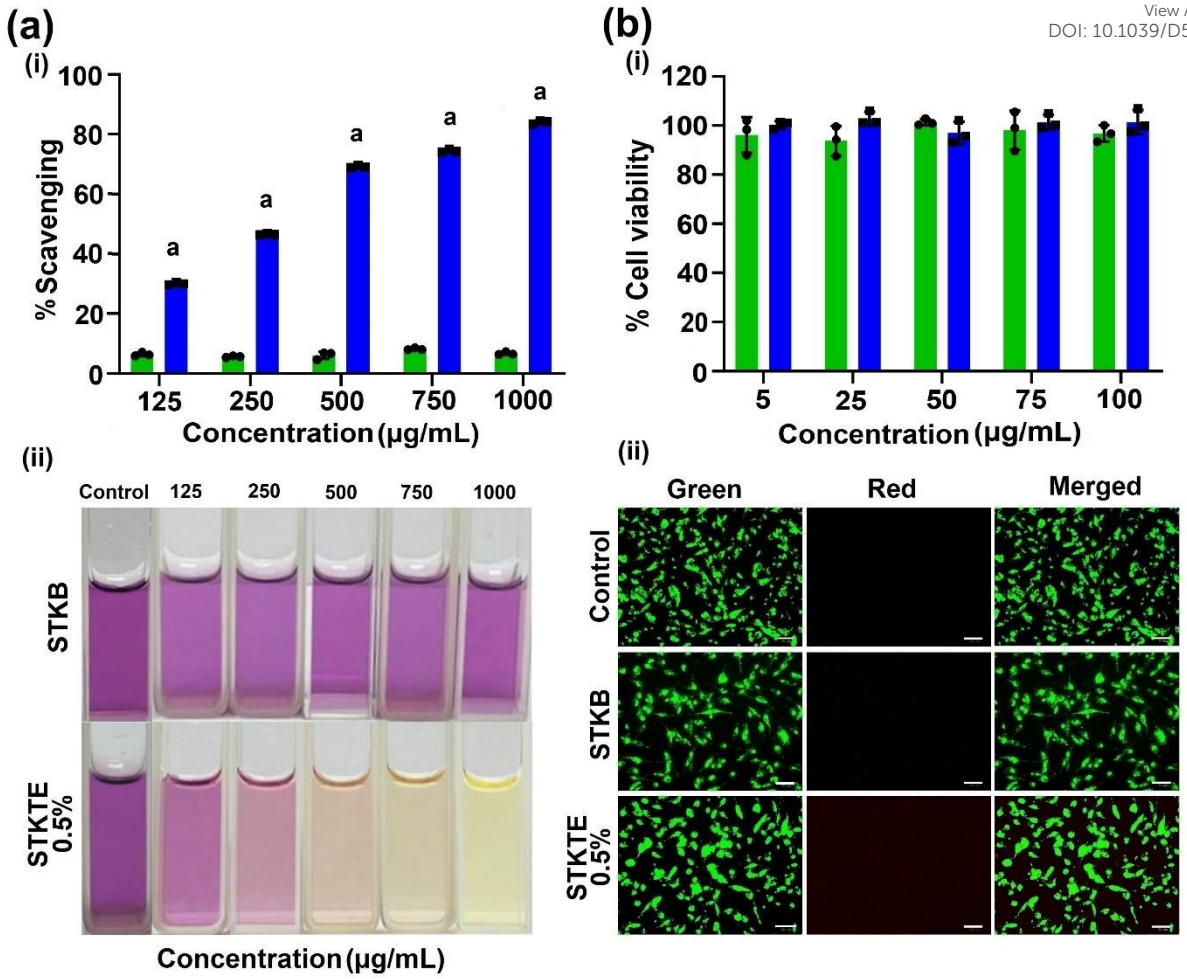

778

779 **Figure 7**

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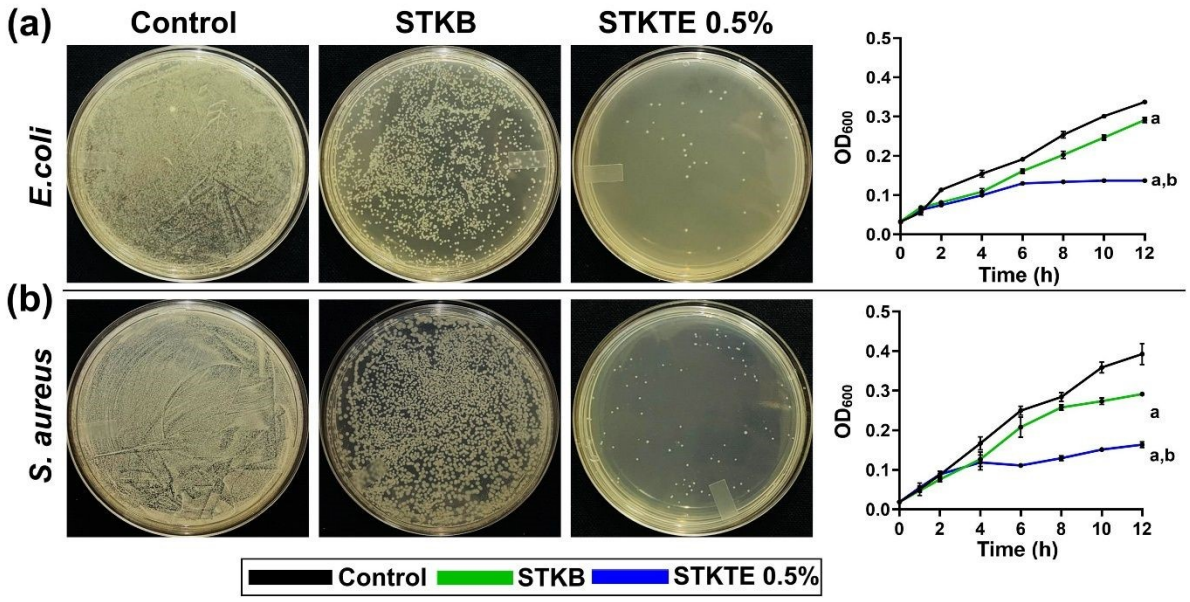

781

782 **Figure 8**

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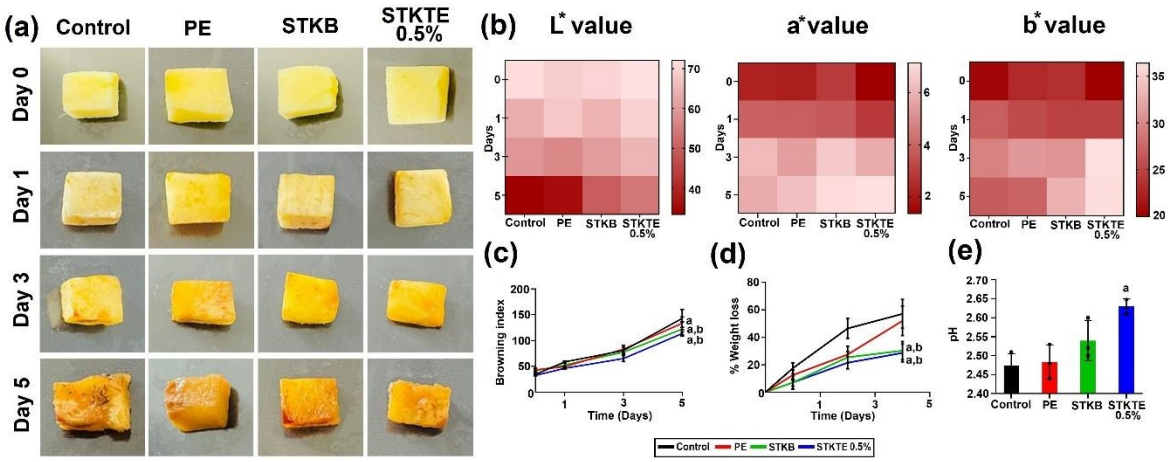

Figure 9

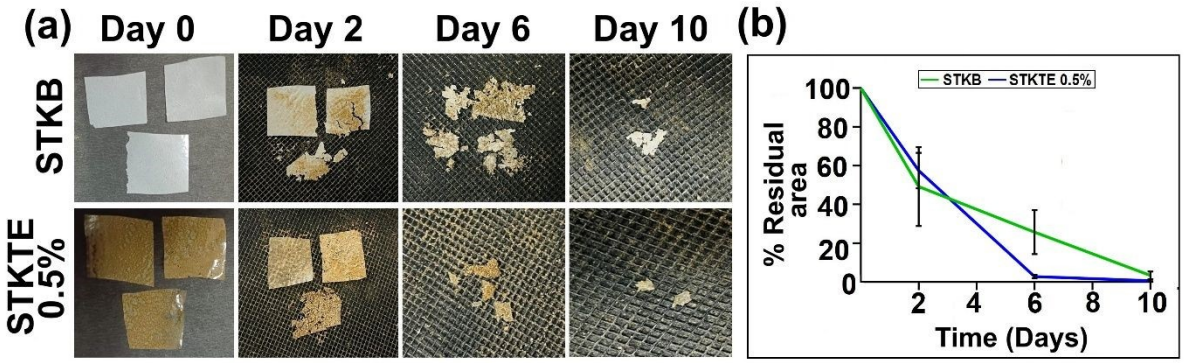

Figure 10

Data Availability Statement

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Data supporting the findings of this study are available within the manuscript.